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DETERMINATION OF RUTIN IN PLANT MATERIALS

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The need for an accurate method for determination of rutin in plant materials has become urgent because of the discovery that this drug restores increased capillary fragility to normal in the human being.[1] In this laboratory an adaptation of the method of Sando and Lloyd,[2] which is based on isolation of the glucoside, was used in early attempts to determine the rutin content of various plant materials. Experience with this procedure has led to modifications from time to time. This paper presents the procedure used at present. In general it is adequate but suffers from the inevitable inaccuracies inherent in isolating and weighing a plant constituent. However, if performed carefully, it is sufficiently precise to afford reliable information for research or control purposes.

The procedure was devised for buckwheat, but it has been used successfully for the analysis of other sources of rutin, such as hydrangea blossoms,[3] forsythia blossoms,[4] black chokecherry leaves,[5] elder, pansy, garden rue, sophora and eucalyptus. Tobacco, which is usually low in rutin, offered some difficulty. The concentrated extracts were sirupy, and the rutin precipitated slowly with a high proportion of impurities. If the fresh plants were extracted with hot strong acetone instead of alcohol, however, the concentrated extracts were more fluid and the rutin crystallized readily with a minimum of impurities. Difficulty was also encountered in the extraction of rutin from the peel of a citrus hybrid.[6]

Rutin in Fresh Plant

Sampling: A field of growing buckwheat is sampled by taking small handfuls (5 to 6 plants) from many places until 5 to 10 pounds are collected. The roots are cut off about 1 inch above the level of the soil.

[1] J. Q. Griffith, Jr., J. F. Couch, and M. A. Lindauer. Effect of Rutin on Increased Capillary Fragility in Man. Proc. Soc. Exptl. Biol. and Med. *55*. 228-229 (1944).

[2] C. E. Sando and J. U. Lloyd. The Isolation and Identification of Rutin from the Flowers of Elder (*Sambucus canadensis* L.). J. Biol. Chem. *58* 737-745 (1924).

[3] J. F. Couch and J. Naghski. Isolation of Rutin from *Hydrangea paniculata* var. *grandiflora* Sieb. J. Am. Chem. Soc. *67*. 1419 (1945).

[4] J. Naghski, W. L. Porter, and J. F. Couch. Isolation of Rutin from Two Varieties of Forsythia. J. Am. Chem. Soc. *69*. 572-573 (1947).

[5] J. F. Couch. The Occurrence of Rutin in a Wild Cherry, *Prunus melanocarpa* (A. Nels.) Rydb. J. Am. Chem. Soc. *70*. 256-257 (1948).

[6] C. F. Krewson and J. F. Couch. Isolation of Rutin from a Citrus Hybrid. J. Am. Chem. Soc. *70*. 257-258 (1948).

Moisture Determination: Place a 50- to 100-gram sample in a tared vessel and dry at 110° C. for 24 hours, cool, and weigh. Calculate loss in weight as percentage of moisture. If the plant is chopped, it is advisable to choose a greater quantity (200 to 300 grams), because it is difficult to obtain a representative sample of such material.

Isolation of Rutin: Select approximately 200 grams of whole sample (weighed to the nearest 0.1 gram) for each rutin determination, and cut into pieces 1 to 2 inches long. Transfer to an extra large Soxhlet extractor as quickly as possible after cutting, using a thin layer of cotton or glass wool to prevent plant material from entering the siphon tube. Immediately add sufficient ethanol (absolute) for extraction (a liter flask requires about 600 ml.) and extract for 2 to 4 hours. At the end of this period, replace the flask containing the ethanol extract with one containing fresh ethanol (to prevent loss of rutin from prolonged heating, since the major portion is extracted in this period) and continue extraction until the extract is colorless (approximately 8 to 12 hours). Combine the extracts in a large casserole and evaporate on a steam bath until all the alcohol is removed (do not evaporate to dryness. Add water if necessary). Add enough water to dissolve the rutin (100 ml. for each 0.4 gram expected), boil vigorously for 1 to 2 minutes and filter through a rapid filter paper (S+S No. 595 black ribbon[7]). Transfer the filter paper to the casserole, boil with a small quantity of water, and refilter. Repeat if necessary until all rutin is dissolved. Store the combined filtrates at room temperature overnight and then in a refrigerator until crystallization is complete (1 to 2 days). Filter through a tared Gooch crucible, wash with cold water, and dry at 110° C. for 4 hours, or to constant weight. Cool, weigh, and calculate as percent crude rutin.

Rutin in Dried Plant

Moisture Determination: Dry a 10-gram sample of ground plant at 110° C. for 4 to 8 hours and calculate loss of weight as percent moisture.

Isolation of Rutin: Place a sample of ground buckwheat containing between 0.5 and 0.75 gram of rutin (15 to 20 grams or 100 grams of low-rutin materials) into a Smalley type extractor,[8] place a plug of cotton on top and bottom of sample layer.[9] Extract with ethyl ether for 8 to 12 hours (to remove fats and carotinoids). Remove the ether by drawing

[7] THE MENTION OF COMMERCIAL PRODUCTS DOES NOT IMPLY THAT THEY ARE ENDORSED OR RECOMMENDED BY THE DEPARTMENT OF AGRICULTURE OVER OTHERS OF A SIMILAR NATURE NOT MENTIONED.

[8] C. O. WILLITS, C. L. OGG, W. L. PORTER AND M. L. SWAIN. DETERMINATION OF RUBBER IN FLESHY AND WOODY TISSUE PLANTS. J. ASSOC. OFF. AGR. CHEM. 29, 370-387 (1946).

[9] A TUBE OF SOME EFFICIENT DESSICANT SHOULD BE PLACED ON TOP OF CONDENSER TO PREVENT MOISTURE FROM ENTERING THE EXTRACTOR. MOISTURE CAUSES THE DRY SAMPLE TO SWELL, AND WHEN SUCTION IS APPLIED TO REMOVE ETHER, THE SAMPLE PACKS IN SUCH A MANNER AS TO PREVENT THE ETHANOL FROM PENETRATING THE MASS DURING RUTIN EXTRACTION.

air through the sample and extract for 4 hours with absolute ethanol. Replace ethanol with fresh ethanol and extract for an additional 4 to 6 hours. Again change the ethanol and continue extraction for a total of 16 hours. Combine the extracts in a casserole and evaporate until the ethanol is removed (do not evaporate to dryness; add water if necessary). Add sufficient water to dissolve the rutin (100 ml. for each 0.4 gram expected), boil vigorously for 1 to 2 minutes (add 0.25 to 0.50 gram of barium chloride if coagulation of non-rutin materials does not occur with boiling) and filter through a rapid filter paper Transfer the filter paper to the casserole and boil with 25 to 50 ml. of water. Filter. Repeat if necessary until all the rutin is dissolved. Determine rutin in filtrates as described under "Fresh Plant."

Correction of Results by Refinement

The crude rutin obtained by this procedure contains 0.5 to 15 percent impurities Consequently the rutin values are usually high, and the wide variation in content of impurities makes it impossible to use a correction factor. However, we have found the method useful in evaluating sources of rutin, agronomic factors influencing rutin in the plant, and processes involved in meal production, and for general control in manufacture of rutin

During the study of factors influencing large scale solvent extraction of fresh plant, several discrepancies appeared which could not be easily explained. The purity of the crude product from large scale production varied considerably with changes in technique; and the purity of the crude product from the analytical procedure varied from sample to sample. A study of the impurities showed that the major portion could be divided into two types--"benzene soluble" and "alcohol insoluble." To correct the crude rutin values, the following purification technique was developed. It is somewhat tedious and time consuming, but is justified where greater accuracy is required. The much simpler method of purification by recrystallization from solvents is unsatisfactory since it involves uncontrollable loss of the glycoside.

Procedure: The Gooch crucible containing the crude rutin is placed in a Soxhlet extractor and extracted with dry benzene for 12 hours. The benzene extract is concentrated to about 10 ml. and transferred to a tared beaker. The extract is then evaporated to dryness on a steam bath, and finally dried at 110° C. for 0.5 to 1 hour. The residue is weighed and calculated as percent "benzene solubles".

The Gooch crucible containing the benzene-extracted crude rutin is dried to remove the benzene, and extracted with boiling absolute ethanol. This is best accomplished by placing the crucible over a suction flask, adding a small quantity of boiling ethanol and triturating the cake of rutin with the flat end of a short stirring rod, being careful not to disrupt the asbestos mat. The solvent is drawn through slowly with vacuum, and the process is repeated several times until all the rutin is dissolved, the volume of solvent being kept as low as possible (40 to 50 ml.). The crucible is then transferred to another suction flask, and

the extract is heated to boiling and filtered through the asbestos mat, in order to recover any asbestos fibers that may have been dislodged and also any insoluble material that may have worked through the mat. The crucible is then rinsed with a few milliliters of fresh solvent to remove any entrained extract, and dried at 110° C. for 2 to 4 hours. The difference between the new weight and the original tare weight is calculated as "alcohol insolubles". Subtract the sum of "benzene solubles" and "alcohol insolubles" from the weight of crude rutin to get the "corrected" weight of rutin.

Crude rutin obtained in large-scale production was corrected in a similar manner. A sample of dry rutin (5 to 7 grams) was weighed into a thimble and extracted with dry benzene, and the benzene soluble material was determined as described above. Then the extracted rutin (freed of benzene) was transferred to a beaker (250 ml.), weighed, and boiled with 100 ml. of absolute ethanol until all rutin was dissolved. The solution was filtered through a tared Gooch crucible, and the residue was washed with several small portions of boiling ethanol. The crucible and contents were dried at 110° C. for 2 to 4 hours and weighed. The alcohol insoluble material was calculated to the original rutin basis.

Discussion

A series of crude rutin samples obtained from various sources was purified by the above-mentioned technique. The results for "benzene solubles" and "alcohol insolubles" are summarized in Table I. The data indicate that because of less efficient filtration the crude rutin from the analytical procedure was more highly contaminated with "benzene solubles" (fats) than the rutin from large-scale production.

The crude rutin from large-scale production has less "alcohol insolubles" than that from analysis of fresh plant but more than that from analysis of dried meal. The crude rutin from large-scale production was more uniform in quality than that from the analytical procedure.

The crude rutin from stems is highly contaminated, especially with "alcohol insolubles," indicating the limitation of the method when applied to materials having a low rutin content.

The importance of correcting the crude rutin value was especially apparent when a critical evaluation was made of production methods.[10] Table II shows selected examples of production runs. When three lots of fresh plant were extracted in the same manner, the yields of crude rutin (based on analytical crude values) ranged from 69 to 77 percent. However, when the crude rutins were purified and the yields recalculated, not only were the three results in close agreement but the values were increased to 81 to 83 percent. The data also show that when dried meal was extracted with isopropanol of different strengths, yields based on

[10] C. F. KREWSON AND J. F. COUCH. PRODUCTION OF RUTIN FROM BUCKWHEAT. IN PREPARATION.

the crude rutin values would have led to erroneous conclusions. Extraction with 55 percent isopropanol gave an apparently high yield; however, the crude product contained 16.9 percent "benzene solubles" and 12.0 percent "alcohol insolubles" and after correction the yeild became the lowest of the series. Rutin prepared by extraction with 65 percent isopropanol was slightly more contaminated and that with 75 percent was slightly less contaminated than the analytical rutin, and, on correction, the changes in yield were small. Crude rutin prepared by extraction with 85 percent isopropanol was so pure that the yield was the lowest of the series, but on correction the yield became the highest.

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TABLE I

VARIATION IN PURITY OF CRUDE RUTIN ISOLATED FROM BUCKWHEAT BY ANALYTICAL PROCEDURE AND BY LARGE-SCALE EXTRACTION

Source	No. of samples	"Benzene solubles"			"Alcohol insolubles"			"Corrected" rutin in plant[1]	
		Min. Percent	Max. Percent	Ave. Percent	Min. Percent	Max. Percent	Ave. Percent	Min. Percent	Max. Percent
FROM ANALYTICAL PROCEDURE									
Dried meal	52	0.02	10.53	1.43	0.0	3.97	1.11	1.11	5.42
Fresh plant	94	.23	13.31	1.61	1.18	15.07	5.94	2.58	5.40
Dried stems	30	.46	9.37	2.51	1.55	72.31	25.08	0.07	0.57
FROM LARGE-SCALE PRODUCTION									
Dried meal	10	.06	0.51 [2](16.92)	0.16	0.45	6.72 [2](12.03)	4.37		
Fresh plant	18	.05	2.75	[3]0.19	1.22	7.79	4.10		

[1] *Moisture-free basis.*

[2] *One sample was obtained by extracting with 55 percent (by vol.) isopropanol; omitted from average.*

[3] *Does not include two high values (2.75 and 1.93 percent) from rutin obtained by extraction with 55 and 65 percent (by vol.) alcohol, respectively.*

CRUDE RUTIN YIELDS AND CORRECTED YIELDS FROM BUCKWHEAT

Moisture in plant Percent	Isopropanol strength Percent (V/V)	Yield of rutin: Crude rutin values: Analysis of plant Percent	Crude rutin values: Large-scale extraction: Percent of plant[1]	Crude rutin values: Large-scale extraction: Percent of analytical value	"Corrected" rutin values: Analysis of plant Percent	"Corrected" rutin values: Large-scale extraction: Percent of plant[1]	"Corrected" rutin values: Large-scale extraction: Percent of analytical value
			FRESH PLANT[2]				
88.8	85	4.3	3.3	77	3.9	3.2	82
88.2	85	4.5	3.1	69	3.6	3.0	83
86.0	85	4.7	3.6	77	4.2	3.4	81
			DRIED LEAF MEAL[3]				
8.1	55	4.6	4.4	96	4.3	3.2	74
8.1	65	4.6	4.4	96	4.3	4.0	93
8.1	75	4.6	4.5	98	4.3	4.2	98
8.1	85	4.6	4.2	91	4.3	4.2	98

[1] *Moisture-free basis.*

[2] *Fifty-pound quantities.*

[3] *Ten-pound quantities.*